



Outbreak of Staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk

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Hummerjohann, Jörg ; Stephan, Roger

Abstract: On October 1, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw cow milk. Within the following 7h, all 14 persons who ingested the cheese fell ill, including 10 children and 4 staff members. Symptoms included abdominal pain and violent vomiting, followed by severe diarrhea and fever. We aim to present this food poisoning outbreak and characterize the causative agent. The duration of the incubation period was dependent of the age of the patient: 2.5h in children under 10 yr of age, 3.5h in older children and teenagers, and 7h in adults. The soft cheese exhibited low levels of staphylococcal enterotoxin (SE) A (>6ng of SEA/g of cheese) and high levels of staphylococcal enterotoxin D (>200ng of SED/g of cheese). Counts of 10(7) cfu of coagulase-positive staphylococci per gram of cheese were detected, with 3 different *Staphylococcus aureus* strains being present at levels >10(6) cfu/g. The 3 strains were characterized using spa typing and a DNA microarray. An enterotoxin-producing strain exhibiting sea and sed was identified as the source of the outbreak. The strain was assigned to spa type tbl 3555 and clonal complex 8, and it exhibited genetic criteria consistent with the characteristics of a genotype B strain. This genotype comprises bovine *Staph. aureus* strains exclusively associated with very high within-herd prevalence of mastitis and has been described as a major contaminant in Swiss raw milk cheese. It is therefore highly likely that the raw milk used for Tomme production was heavily contaminated with *Staph. aureus* and that levels further increased due to growth of the organism and physical concentration effects during the cheese-making process. Only a few staphylococcal food poisoning outbreaks involving raw milk products have been described. Still, in view of this outbreak and the possible occurrence of other foodborne pathogens in bovine milk, consumption of raw milk and soft cheese produced from raw milk constitutes a health risk, particularly when young children or other members of sensitive populations are involved

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INTERPRETIVE SUMMARY

Outbreak of Staphylococcal Food Poisoning among Children and Staff at a Swiss Boarding School Due to Soft Cheese Made from Raw Milk

Johler

In October 2014, an outbreak of food poisoning due to consumption of Tomme soft cheese made from raw cow milk affected ten children and four staff members at a Swiss boarding school. Low levels of staphylococcal enterotoxin A and high levels of staphylococcal enterotoxin D were detected in the cheese. The *Staphylococcus aureus* strain identified as the source of the outbreak was assigned to genotype B, a genotype associated with high within-herd prevalence of bovine mastitis that was reported to represent a major contaminant in Swiss raw milk cheese.

STAPHYLOCOCCAL FOOD POISONING DUE TO RAW MILK CHEESE

**Outbreak of Staphylococcal Food Poisoning among Children and Staff at a Swiss Boarding
School Due to Soft Cheese Made from Raw Milk**

**Sophia Johler^{*,1}, Delphine Weder[†], Claude Bridy[†], Marie-Claude Huguenin[†], Luce Robert[†],
Jörg Hummerjohann [‡], Roger Stephan^{*}**

^{*}Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, 8057 Zurich,
Switzerland

[†]Service de la Consommation et des Affaires Vétérinaires, Département du Développement
Territorial et de l'Environnement République et Canton de Neuchâtel, 2001 Neuchâtel,
Switzerland

[‡]and Agroscope, Institute for Food Sciences, 3003 Bern³, Switzerland

¹Corresponding author: Sophia Johler, Institute for Food Safety and Hygiene, Vetsuisse Faculty
University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland, Phone: +41-446358651,
Fax: +41-446358908, E-mail: sophia.johler@uzh.ch

ABSTRACT

On October 1st, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw cow milk. Within the following seven hours, all 14 persons that had ingested the cheese fell ill, among them ten children and four members of the staff. Symptoms included abdominal pain and violent vomiting, followed by severe diarrhea and fever. The duration of the incubation period was dependent of the age of the patient, with 2.5 hours in children under 10 years of age, 3.5 hours in older children and teenagers, and 7 hours in adults. The soft cheese exhibited low levels of staphylococcal enterotoxin A (> 6 ng SEA/g cheese) and high levels of staphylococcal enterotoxin D (> 200 ng SED/g cheese). A total of 10^7 CfU coagulase-positive *Staphylococci* per gram cheese were detected, with three different *Staphylococcus (Staph.) aureus* strains being present at levels higher than 10^6 CfU/g. The three strains were characterized using *spa* typing and a DNA microarray. An enterotoxin-producing strain exhibiting *sea* and *sed* was identified as the source of the outbreak. The strain was assigned to *spa* type t711 and clonal complex 8 and exhibited genetic criteria consistent with the characteristics of a genotype B strain. This genotype comprises bovine *Staph. aureus* strains exclusively associated with very high within-herd prevalence of mastitis and has been described as a major contaminant in Swiss raw milk cheese. It is therefore highly likely that the raw milk used for Tomme production was strongly contaminated with *Staph. aureus* and that levels further increased due to growth of the organism and physical concentration effects during the cheese making process. Only few SFP outbreaks involving raw milk products have been described. Still, in view of this outbreak and the possible occurrence of other foodborne pathogens in bovine milk, consumption of raw milk and soft cheese produced from raw milk constitutes a health risk, in particular when young children or other members of sensitive populations are involved.

61 Key words: Outbreak investigation, *Staphylococcus aureus*, raw milk cheese, genetic
62 characterization, genotype B

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INTRODUCTION

Staphylococcal Food Poisoning (SFP) is one of the most prevalent causes of food-borne intoxication worldwide, resulting in an estimated 241'148 cases and six deaths in the US alone (Scallan et al., 2011). After a short incubation period of 2-6 hours, patients exhibit nausea, followed by violent vomiting and diarrhea (Tranter, 1990). As clinical symptoms are typically self-limiting, only 10% of SFP patients are estimated to visit a hospital (Holmberg and Blake, 1984). Consequences include pronounced under-reporting of the disease and scarce scientific data on the characteristics of *Staph. aureus* strains causing SFP.

SFP is caused by consumption of staphylococcal enterotoxins (SEs) preformed by *Staph. aureus* in food. To date, more than 20 different SEs and SE-like superantigens have been described (Hennekinne et al., 2012), but only a few were demonstrated to elicit an emetic response in a monkey feeding assay. SEs that were shown to exhibit emetic activity include the classical enterotoxins SEA/SEB/SEC/SED/SEE, and to a limited degree also newly described enterotoxins (Thomas et al., 2007).

In SFP outbreak investigations, identification of the causative strain can be challenging, as SEs are highly heat-resistant. Even when the organism was inactivated and can therefore no longer be isolated from a food item, the highly stable enterotoxins preformed by *Staph. aureus* can still cause SFP (Le Loir et al., 2003). In addition, identification of the causative strain in an outbreak investigation is aggravated by the high prevalence of *Staph. aureus* in humans and animals. *Staph. aureus* persistently colonizes the anterior nares of 20-30% of the human population (van Belkum et al., 2009), causes a multitude of infections in humans and livestock, and can be isolated from a wide range of food items (Baumgartner et al., 2014). The organism also represents a common cause of bovine mastitis and can be detected in bulk tank milk at prevalence rates of 27-42% (Oliver et al., 2009).

On October 1st, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw milk. Within the following seven hours, all 14 persons that had consumed the cheese fell ill, among them ten children and four members of the staff. Based on the short incubation time, as well as the clinical symptoms, staphylococcal food poisoning due to consumption of the raw milk cheese was considered a possible cause of the outbreak.

MATERIALS AND METHODS

Enumeration of Coagulase-Positive Staphylococci

Coagulase-positive *Staphylococci* (CPS) present in the Tomme soft cheese were enumerated using the plate count technique on rabbit plasma fibrinogen agar (RPF, Oxoid, Basel, Switzerland).

CPS Isolation, Cell Lysis and DNA Extraction

Different morphologies of colonies forming an opaque fibrin halo on RPF after 48 hours of incubation at 37°C were subcultured on sheep blood agar and incubated overnight at 37°C. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and following the manufacturers' instructions.

Spa Typing

The polymorphic X region of the *spa* gene was determined as previously described (Wattinger et al., 2012). Briefly, *spa* was amplified using *spa*-1113f and *spa*-1514r primers (Table 1) and the GoTaq PCR system (Promega AG, Dübendorf, Switzerland) at the following reaction conditions: (i) 5 min at 94°C; (ii) 35 cycles of 45 s at 94°C, 45 s at 60°C and 90s at 72°C; and (iii) 10 min at 72°C. PCR amplicons were purified using the MinElute PCR

Purification Kit (Qiagen, Hilden, Germany). Sequencing was outsourced (Microsynth, Balgach, Switzerland) and *spa* types were determined using the *spa*-server (<http://spa.ridom.de/>) (Harmsen and Claus, 2003).

Microarray Based Genotyping

The *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany) was used to further characterize the *Staph. aureus* strains. This analytical tool can be used to predict clonal complex assignment (Monecke et al., 2008) and to determine the presence/ absence of over 300 resistance and virulence genes including genes encoding major SEs (*sea-see*), newly-described SEs (*seg, seh, sei, sej, sek, sel, seq, ser*) and enterotoxin-like superantigens (*selm, seln, selo, selu*).

Detection of Staphylococcal Enterotoxins in Cheese

To screen for all major SEs, an extract of the cheese sample was prepared and analyzed using SET2 miniVIDAS (bioMérieux, Lyon, France) according to the manufacturer's recommendations. The SET-RPLA kit (Oxoid) was subsequently used to enable semiquantitative detection of SEA, SEB, SEC, and SED.

RESULTS AND DISCUSSION

All persons that had consumed the raw milk cheese fell ill, among them ten children and four members of the staff (Table 2). While the average incubation time was 4.4 hours, the individual duration of the incubation period was dependent of the age of the patient. Only 2.5 hours after consumption of the cheese, the two youngest children (age 8 and 9) complained about abdominal pain, ague, and aching limbs that progressed quickly to emesis, followed by severe diarrhea, and

fever. One hour later, the older children (age 10-16) exhibited the same symptoms, followed 3.5 hours later by the adults (age 31-57). One person sought medical care and was treated. We detected a total of 10^7 CFU coagulase-positive *Staphylococci* per gram cheese in the Tomme sample. Different morphologies of coagulase-positive colonies exhibiting a phenotype consistent with *Staph. aureus* were visible on RPF agar (Figure 1), indicating contamination of the product with more than one *Staph. aureus* strain. Using SET2 miniVIDAS to screen for major SEs, the cheese tested positive for SEA-SEE in 25 g of product. Subsequently, the SET-RPLA kit was used for semiquantitative detection of SEA, SEB, SEC, and SED, identifying low levels of SEA (> 6 ng SEA/g cheese) and high levels of SED (> 200 ng SED/g cheese) in the Tomme soft cheese. SEA is the most common SE recovered from food-poisoning outbreaks (78%) and was reported to cause symptoms of intoxication in humans at a total dose of only 200 ng SEA (Balaban and Rasooly, 2000). In a monkey feeding assay, 25 μ g SEA /kg body weight induced emesis and in the house musk shrew, the 50% emetic dose (ED_{50}) for peroral administration equaled 32 μ g SEA /kg body weight (Hu and Nakane, 2014). Although SED represents the second most common SE and can be detected in 38% of SFP outbreaks (Balaban and Rasooly, 2000), there is no comparable data on the effect of SED after oral intake. However, intraperitoneal injection of a total dose of 40 μ g SED was shown to have an emetic effect in the house musk shrew (Hu and Nakane, 2014).

Three different *Staph. aureus* strains (SA_1, SA_2, SA_3) were isolated from the cheese and were further characterized by *spa* typing (Wattlinger et al., 2012) (Table 3). All three strains were present in the cheese sample at levels higher than 10^6 CFU/g, with SA_1 occurring most frequently. DNA microarray analysis was used to identify the *Staph. aureus* isolate that had produced the major SEs previously detected and to generate a virulence and resistance gene profile of the strains. Major staphylococcal enterotoxin genes were only detected in SA_1, a

strain that exhibited both *sea* and *sed* (Table 3). SA_1 was assigned to CC8, a clonal complex frequently detected among strains isolated from humans, animals, and food products. Based on the enterotoxins SEA and SED detected by SET-RPLA and the enterotoxin genes *sea* and *sed* detected in the microarray, *Staph. aureus* strain SA_1 isolated from the Tomme raw milk cheese was identified as the source of the outbreak.

SA_1 was assigned to *spa* type t711 and clonal complex 8. The *spa* type t711 is commonly detected among bovine mastitis isolates in Switzerland (Sakwinska et al., 2011; Johler et al., 2011) and has also been described in association with infections in humans, including MRSA infections caused by the USA300 clone (Yabe et al., 2010). While SA_1 exhibited genes involved in beta lactam resistance (*blaZ*, *blaI*, *blaR*), no genes conferring methicillin resistance were detected. SA1 belongs to CC8, a clonal complex frequently linked to SFP outbreak strains and staphylococcal infections in humans and animals (Wattlinger et al., 2012; Resch et al., 2013; Monecke et al., 2009). In Switzerland, 13-36% of the *Staph. aureus* isolated from bovine mastitis milk (Moser et al., 2013; Sakwinska et al., 2011) and 12% of the *Staph. aureus* isolated from ready-to-eat foods (Baumgartner et al., 2014) belong to CC8.

SA_1 exhibits genetic criteria (*sea*, *sed*, *sej*, CC8) consistent with the characteristics of a genotype B strain (Moser et al., 2013; Boss et al., 2011). This genotype comprises bovine *Staph. aureus* strains exclusively associated with very high (up to 65%) within-herd prevalence of mastitis (Graber et al., 2009) and has been described as a major contaminant in Swiss raw milk cheese (Hummerjohann et al., 2014). It is therefore highly likely that the raw milk used for Tomme production was strongly contaminated with *Staph. aureus*. During the production process of soft cheese made from raw milk, *Staph. aureus* levels can further increase due to both growth of the organism, as well as physical concentration effects that result in an estimated increase of 1 log₁₀ (Peng et al., 2013).

Only few SFP outbreaks involving raw milk products have been described and many of these were associated with goat or sheep milk products rather than products made of bovine raw milk (De Buyser et al., 2001; Ostyn et al., 2010; Giezendanner et al., 2009). Still, in view of this outbreak and the possible occurrence of other foodborne pathogens such as Shigatoxin-producing *Escherichia coli* in bovine milk, consumption of raw milk and soft cheese produced from raw milk constitutes a health risk, in particular when young children or members of other sensitive populations are involved.

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TABLES

Table 1: Primers used for *spa* typing

Name	Nucleotide sequence	Reference
spa-1113f	5'-TAA AGA CGA TCC TTC GGT GAG C-3'	(Aires-de-Sousa et al., 2006)
spa-1514r	5'-CAG CAG TAG TGC CGT TTG CTT-3'	(Aires-de-Sousa et al., 2006)

Table 2: Overview of the ten children and four staff members that suffered from clinical signs of SE intoxication. With an average incubation time of 4.4 hours, the onset of symptoms was earlier in children than in adults

Patient	Sex	Year of Birth	Incubation time in hours	Symptoms
P1	M	2006	2.5	Abdominal pain, emesis, diarrhea, fever
P2	M	2005	2.5	Abdominal pain, emesis, diarrhea, fever
P3	M	2004	3.5	Abdominal pain, emesis, diarrhea, fever
P4	M	2001	3.5	Abdominal pain, emesis, diarrhea, fever
P5	M	2001	3.5	Abdominal pain, emesis, diarrhea, fever
P6	M	2000	3.5	Abdominal pain, emesis, diarrhea, fever
P7	M	1999	3.5	Abdominal pain, emesis, diarrhea, fever
P8	F	1999	3.5	Abdominal pain, emesis, diarrhea, fever
P9	F	1999	3.5	Abdominal pain, emesis, diarrhea, fever
P10	M	1998	3.5	Abdominal pain, emesis, diarrhea, fever

P11	M	1983	7	Abdominal pain, emesis, bloody diarrhea, fever
P12	M	1981	7	Abdominal pain, emesis, diarrhea, fever
P13	M	1973	7	Abdominal pain, emesis, diarrhea, fever
P14	F	1957	7	Abdominal pain, emesis, diarrhea, fever

Table 3: Characteristics of the three *S. aureus* strains isolated from the Tomme soft cheese made of raw milk, including the presence of genes encoding staphylococcal enterotoxins (SE)

ID	Major SE genes					Genes encoding newly described SEs and SE-like superantigens	<i>spa</i> ¹	<i>agr</i> type	CC ²
	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>	<i>see</i>				
SA_1	+	-	-	+	-	<i>sej, ser</i>	t711	agrI	CC8
SA_2	-	-	-	-	-	<i>egc</i> cluster (<i>seg, sei, selm, seln, selo, selu</i>)	t018	agrII	CC705
SA_3	-	-	-	-	-	<i>egc</i> cluster (<i>seg, sei, selm, seln, selo, selu</i>)	t458	agrI	CC20

¹⁾ Assignment to *spa* types.

²⁾ Assignment to clonal complexes based on DNA microarray predictions.

FIGURES

Figure 1: RPF agar used for enumeration of coagulase-positive Staphylococci. Different morphologies of colonies consistent with a *Staph. aureus* phenotype were visible on RPF (dilution 1:1'000'000), indicating contamination of the product with more than one *S. aureus* strain